IV. EXPERIMENTAL RESULTS

The present study was undertaken in order to analyze the physical and chemical mutagenesis on Green gram [Vigna radiata (L.) Wilczek] variety VBN2 was subjected to different mutagenic dose/concentration for induction of mutation. There were two mutagens namely, physical mutagen (gamma rays) and chemical mutagen (Ethyl methane sulphonate) used on seeds as biological material. The experiment was conducted in M₁, M₂, M₃ and M₄ generations. The results obtained on growth, yield, biochemical, cytological and molecular level variations were given below:

4.1. M₁ GENERATION

Laboratory studies

Seeds were treated with different dose/concentration of mutagens were grown in the petri plates with five replication. Based on the germination percentage, the LD₅₀ value was calculated in all physical and chemical mutagens dose/conc. along with control. Based on the germination studies, 50 % lethality showed at 40 kR gamma rays (49.00 %) and 20 mM EMS (50.50 %) (Table-1 and 2).

4.2. Field studies

Seedling height on the 7th day (cm/seedling)

Among the different mutagens, seedling height was decreased with increased dose/conc. in which EMS (6.44 cm) showed more declination than other mutagens and control (19.20 cm) plant (Table-3; Plate-I).
Plant survival (%) on 30th day

All the mutagenic dose/concentration, the control plants showed maximum survivability when compared to physical and chemical mutagens. The 50 mM EMS treated plants showed more reduction (79.05 %) and 40 mM EMS (77.10 %) due to high toxic concentration when compared to all other gamma rays treatments (Table-4).

4.3. Quantitative traits of mean performance (At maturity)

In this study, various quantitative mean performances in M1 generation were recorded in all mutagenic concentration which was tabulated in sequence.

All the mutagenic concentration showed a gradual reduction of mean performance than the control with increasing dose/conc. in the M1 generation. The range, mean, standard error and percent reduction over control are presented in Table 6-10. In figures, the values are expressed as Mean±SE for each group.

Plant height (cm/plant)

Among the different dose/conc. of mutagens, plant height showed decreasing trend of mean values than control in M1 generation. The highest reduction was recorded at 50 mM EMS (27.93 cm) followed by 50 kR gamma rays (30.66 cm) when compared to other dose/conc. and control (51.33 cm) plants (Table-6).

Number of branches per plant: A gradual reduction of mean performance was noticed in number of branches per plant for all the mutagenic concentration when compared with control. Among them, the highest reduction was observed at 50 mM
EMS (2.90) followed by 40 kR gamma rays (3.26) treated plants than control plants (4.26) and other treatments (Table-6).

**Number of leaves per plant**

With different dose/conc. of mutagens, declination of mean values was recorded in number of leaves in M₁ generation. In this generation, the highest reduction of leaves was noted in 50 mM EMS (24.16), 40 mM EMS (25.60) when compared to other EMS, gamma rays and control plants (38.26) (Table-7).

**Days to first flowering**

A gradual increasing of days to flowering was observed at all the mutagen treated plants than control. Among them, 50 mM EMS treatment was taken more days (35.00 days) for flowering whereas, in control it was 32.80 days (Table-7).

**Number of pod cluster per plant**

Treatments differed significantly in the development of pod clusters. Among the different dose/conc., the reduction of mean values 50 mM EMS (7.16) followed by 40 mM EMS (8.20) than the control plants (13.23) (Table-8).

**Number of pods per plant**

The mean performance of number of pods per plant showed a gradual declination among different dose/conc. of mutagens when compared to control. The
highest reduction of pods was noted at 50 mM EMS (11.00), 50 kR gamma rays (13.00) than other mutagenic treatments and control plant (30.80) (Table-8).

**Number of seeds per pod**

The mean performance on number of seeds per pod showed a gradual declination among the different dose/concentration of mutagens when compared to control. The higher reduction of number of seeds per pod was recorded at 50 mM EMS (4.16) than other mutagenic dose/conc. and control plant (10.33) (Table-9).

**Hundred Seed weight (g)**

There was slight reduction of hundred seeds weight was recorded at all the mutagenic dose/concentration when compared to control. However, EMS treatments showed more reduction particularly at 50 mM EMS (2.22 g) and 40 kR gamma rays (2.16 g) showed more reduction than the other mutagenic dose/concentration treated plant (Table-9).

**Seed yield per plant**

In general, reduction of seed yield was recorded at all the mutagenic concentration when compared to control. Among them, 50 mM EMS (5.19 g) showed higher reduction of seed yield per plant than other physical and chemical treated plants and control (11.82 g) (Table-10).
Protein content (%) 

In M₁ generation, mean performance of protein content showed a gradual decline among different dose/concentration of mutagens when compared to control. The higher reduction of protein content was obtained at 50 mM EMS (20.32 %) than other mutated plants and control (22.60 %) (Table-10).

4.4. M₂ GENERATION

Field observations 

The M₂ generation plants were raised from the M₁ seed basis with three replications for different mutagenic dose/concentration. The M₂ seedlings were examined for identification of chlorophyll mutants (up to 30th day) and viable mutants (up to maturity), which was helpful to analyze the mutants frequency, effectiveness and efficiency of mutagens.

Observation of chlorophyll and viable mutants 

Micro and macro-mutants play an important role to assess the concentration of mutagens. Almost all the mutagenic treatments showed different degree of mutants with respective treatments.

In the present investigation, some of the chlorophyll and viable mutants were observed in the different concentration of mutagen treated plants. They were chlorina, albino, xantha, viridis and tall, dwarf, bushy, oval leaf, narrow leaf, early maturity, late maturity, short pod, long pod, non-hairy pod, bold size seed and small size seed mutants (Table-11; Plates II - IV).
**Frequency of chlorophyll and viable mutants**

Based on the number of mutants observed in the various mutagens, mutation frequency was calculated. The frequency was increased with increasing concentration of mutagen up to a certain level. Thereafter, it was reduced due to heavy concentrations. Among the physical and chemical mutagens, EMS provided more number of chlorophyll and viable mutants followed by gamma rays treatments. The high frequency of chlorophyll and viable mutants was recorded at 20 mM EMS treatment (11.50) followed by 40 kR gamma rays (8.72) treated plants (Table-11). Whereas other mutagenic dose/concentration produce minimum number of chlorophyll and viable mutants.

**4.5. Effectiveness and Efficiency**

Effectiveness and efficiency are important features for selection the optimal dose/concentration in plant breeding. In the present study, these criteria were calculated based on lethality and injury of the plants at 30\textsuperscript{th} day. The minimum level of mutation frequency was observed in 50 kR gamma rays (5.88) and 30 mM EMS treated mutants (6.66). The effectiveness of mutagens was maximum in 20 mM EMS (21.80) followed by 40 kR gamma rays (19.16) (Table-12). Whereas efficiency was more at 40 kR gamma rays on both lethality and injury (19.67 and 34.78) basis followed by 20 mM EMS (17.68 and 33.37) treated plants. The effectiveness and efficiency was inconsistent in nature among the mutagenic treatments in the present study.
4.6. Quantitative mean performance in M₂, M₃ and M₄ generations

Field observation (At maturity)

Among the both mutagens with various dose/concentrations, a gradual increase of mean values was observed at certain level in the M₂, M₃ and M₄ generation. Therefore, higher mutagenic concentration showed decreasing trend of mean performance than that of other mutagenic dose/conc. and control. So, the study, the desired concentration of gamma rays and EMS were selected for further studies. The ascending and descending dose/concentration of LD₅₀ values was selected to develop M₂, M₃ and M₄ generations for crop improvement.

Plant height (cm/plant)

A gradual increase of mean for plant height was noticed with increasing dose/conc. of mutagens in M₂, M₃ and M₄ generations than that of control. Among the higher mean for plant height was observed in M₂ generation at 20 mM EMS (59.20 cm) followed by 40 kR gamma rays (56.72 cm). This was significantly increased than other concentration and control. Whereas, 30 EMS and 50 kR gamma rays showed reduction in plant height than control (49.93 and 47.73 cm) (Table-13).

In M₃ and M₄ generations showed increased mean performance of plant height when compared to M₁ and M₂ generations with some exceptions.
Number of branches per plant

The mean for primary branches was showed a gradual increase in LD_{50} dose/concentration than control. It was higher at 40 kR gamma rays (4.93) followed by 20 mM EMS (4.70) when compared to control (4.56) values in M_{2} generation (Table-14).

The mean performance on maximum number of branches based on the different dose/conc. In M_{3} generation, the maximum number of branches was developed in 40 kR gamma rays (5.12) and 20 mM EMS (4.71) treated plants than the other mutagenic treatments and control. But in M_{4} generation plants showed with some exceptions.

Number of leaves per plant

Among the various dose/concentration of mutagens, 40 kR gamma rays (46.64) showed more number of leaves followed by 20 mM EMS (43.98) treated plants (Table-15).

Due to the response of mutagenic treatments, the number of leaves per plant was high in M_{3} and M_{4} generation in treated plants than the M_{1} generation.

Days to first flowering

Different mutagens with various dose/concentration of M_{2} generation showed slight level of decreasing in number of days for first flowering. Among them, 20 mM EMS (28.00 day) showed elimination of days for flowering than 40 kR gamma rays
(29.34 day). These mean performances showed lesser number of days was taken for blooming than the control plant (31.16 day) (Table-16).

**Number of pod cluster per plant**

Among the various dose/concentration of mutagens, the pod clusters maximum in 20 mM EMS treatments (18.30 and 19.87) than 40 kR gamma rays (17.42 and 17.73) treatments at M₂ and M₃ generations, respectively. Above mentioned mean performance was slightly increased number of pods than control (13.36 and 17.60) M₂ and M₃ generations, respectively (Table-17).

**Number of pods per plant**

The data on number of pods per plant was noticed with increasing dose/conc. of mutagens up to certain level. Among them, higher mean values for pods was observed at 20 mM EMS (26.46) followed by 40 kR gamma rays (25.43) plants. This was significantly increased than other dose/conc. and control plants (22.36) (Table-18).

In M₂ and M₃ generations showed maximum mean performance on number of pods in 20 mM EMS and 40 kR gamma rays with some exceptions than control plants (Table-18).

**Number of seeds per pod**

The results pertaining to number of seeds per pod were presented in Table-19. The results indicated that higher number of seeds in 40 kR gamma rays (11.56) followed by 20 mM EMS (11.47) than control (10.41) plants. Besides, other
dose/conc. particularly, the LD$_{50}$ concentration showed slight increase in number of seeds in $M_3$ and $M_4$ generations.

**Hundred Seed weight (g)**

The data pertaining to the effect of mutagens on hundred seed weight of green gram plants are showed in Table-20. In all the mutagenic treatments, hundred seed weight was gradually increased in 20 mM EMS (4.85 g) and 40 kR gamma rays (4.63 g) when compared to control (4.00 g) plants.

**Seed yield per plant (g)**

The Table 21 exhibits that the seed content of green gram was significantly affected by different dose/conc. of physical and chemical mutagen treated plants. There was slight variation found among the both mutagens with various concentrations in the present study. In $M_2$ generation, among the various treatments, 20 mM EMS (11.81 g) yield more number of seeds followed by 40 kR gamma rays (11.72 g) than control (10.26). Whereas, higher dose/concentration showed less in number of seeds than the above mentioned mutagenic treatments. In $M_3$ and $M_4$ generations had also the same results.

**Protein content (%)**

Due to the induction of mutagenic treatments, protein content was significantly higher in 20 mM EMS treatments (24.02 %) than gamma rays and control plants in $M_2$ generation. Similarly, $M_3$ and $M_4$ generations, the high level of protein content was produced same EMS concentration followed by gamma rays
(40 kR) than control plants. Whereas, higher dose/conc. of gamma rays and ethyl
methane sulphonate mutagens treated plants decreased quantity of protein than
control plants (Table-22).

4.7. VARIABILITY ANALYSIS

Phenotypic and genotypic co-efficient variation (PCV, GCV),
heritability (h^2) and genetic advance as per cent of mean (GA %)

Knowledge about the existence genetic variation and the association between
various plant characters and their heritability is important for assessing the
potentiality of plants to respond to plant improvement programme. This depends upon
the nature and magnitude of variability as well as other genetic parameters of
important quantitative characters.

In the present investigation, genetic variability was analyzed with effect of
gamma rays and Ethyl methane sulphonate on green gram in M_2, M_3 and M_4
generation.

Genetic variability analyses in M_2, M_3 and M_4 generations

PCV, GCV, h^2 and GA (%)

Plant height (cm/plant)

Among the various quantitative traits, plant height showed high PCV and
GCV. The moderate to high phenotypic and genotypic co-efficient of variation were
recorded in M_2 generation with effect of gamma rays and EMS. While, high
dose/conc. produced moderate and low level of PCV and GCV values.
In M₃ and M₄ generations generated high heritability and genetic advance values particularly at 20 mM EMS (63.68; 64.20) than gamma rays treated plants. Whereas moderate values observed at lower concentration (Table-13a).

**Number of branches per plant**

The genotypic co-efficient variation values were moderate in 20 mM EMS (26.60; 27.82 and 25.48 %) followed by 40 kR gamma rays (24.57; 25.74 and 24.61) in M₂, M₃ and M₄ generations, respectively.

Among the concentrations, 20 mM EMS (59.82) in M₄ generation and showed more heritability. While genetic advance as per cent of mean was higher at the LD₅₀ of mutagens (Table-14a).

**Number of leaves per plant**

Among the mutagens, moderate PCV and GCV were observed at 20 mM EMS (25.20; 23.90) followed by 40 kR gamma rays (23.17; 21.87).

Heritability showed high values in 20 mM EMS (69.31) and 40 kR gamma rays (65.10). While high concentration of gamma rays and EMS showed moderate and low level of heritability. Whereas, high genetic advance as per cent of mean recorded at LD₅₀ dose/conc. (Table-15a).

**Days to first flowering**

In M₂ generation, the moderate PCV and GCV were recorded at 20 mM EMS (18.29; 14.51). Whereas, other gamma rays and EMS dose/concentration showed low
PCV and GCV values. Among the various dose/conc. of physical and chemical mutagens, high heritability was recorded at 20 mM EMS (73.88) followed by 40 kR gamma rays (70.22) treated plants in M₄ generation. While, higher heritability value was recorded at all the dose/conc. of mutagens under physical and chemical mutagenic treatments in days to first flowering parameter. Whereas, high genetic advance as per cent of mean was recorded at 20 mM EMS (46.67) followed by 40 kR gamma rays (44.54) in M₄ generation. But high concentration of gamma rays and EMS showed moderate level of genetic advance as per cent of mean (Table-16a).

**Number of pod cluster per plant**

Variability was more in number of pod cluster per plant at all the mutagenic treatments. Among them, M₃ generation plants produced high level of PCV particularly at 20 mM EMS treatments (32.64) and 40 kR gamma rays (30.55) treatments.

In M₄ generation plants generated maximum level of heritability at 20 mM EMS (81.50) followed by 40 kR gamma rays (74.60) plants when compared to all other dose/conc. and generations. Besides heritability, genetic advance as percent of mean was also produced EMS treated plants (Table-17a).

**Number of pods per plant**

Among the various dose/concentration, high PCV obtained at 20 mM EMS (30.11) followed by moderate level of PCV and GCV were obtained at all other mutated populations.
Number of pods per plant was produced maximum heritability in M₄ generation EMS treatments particularly at 20 mM EMS (82.48) when compared to gamma rays treatments. Whereas, genetic advance as per cent of mean was showed high at 20 mM EMS (45.65 %) followed by 40 kR gamma rays (43.20 %) treatments (Table-18a).

**Number of seeds per pod**

Moderate level of PCV and GCV were obtained at 40 kR gamma rays (27.30; 24.47) and 20 mM EMS treatments (27.16; 24.69) than the other treatments.

Heritability of gamma rays and EMS produced high level in all these generations. Among them, 20 mM EMS (76.33 %) produced high heritability in this study. While, the genetic advance as per cent of mean was high at all the dose/conc. with few exceptions (Table-19a).

**Hundred Seed weight (g)**

Due to the induction of different mutagenic dose/concentration, moderate PCV and GCV results were observed at 20 mM EMS (27.65; 24.90) in M₃ generation followed by 20 mM EMS (25.82; 23.74) treatments in M₄ generation. While, high dose/conc. showed moderate and low level of PCV and GCV.

Heritability was high at 20 mM EMS (70.19) under M₃ population followed by 40 kR gamma rays produced at (62.76 %). Whereas, genetic advance as per cent of mean was much higher at 20 mM EMS (50.31) and 40 kR gamma rays (49.20) treatments (Table-20a).
Seed yield per plant (g)

Moderate PCV and GCV were noted in 20 mM EMS (26.28; 24.11) under M4 populations followed by 40 kR gamma rays (26.22; 24.53). While, 30 mM EMS at gave the minimum value of PCV and GCV (20.19; 19.16) when compared to all other treatments and generations in seed yield producing parameter.

Among the different dose/conc., the better performance of heritable characters developed at 20 mM EMS (71.59% and 70.22%) in M2 and M3 generations, respectively. While genetic advance as per cent of mean was also higher at all dose/conc. of mutagens with some exceptional case (Table-21a).

Seed protein content (%)

Table 22a represented the effect of induced physical and chemical mutagens on the protein content of green gram in M2, M3 and M4 populations. All these generations, produced moderate level of PCV and GCV. The high amount of heritable characters produced at 20 mM EMS (78.90; 75.41 and 72.89%) treated plants than the other treatments respectively. The genetic advance as per cent of mean was produced high level variations with few exceptions.

4.8. Cytological Analysis

Cytological studies provide more information regarding the response of green gram genotype to a particular mutagen and provide greater chances for the selection of desired characters. Root mitotic studies revealed a wide range of chromosomal aberration such as stickiness, precocious movement, anaphasic laggard, anaphasic
single, double and multiple bridges. The chromosome studies were made in treated (M_1 and M_2 generation plants) and control. Chromosome bridges and laggards were observed for all mutagenic treatments. Maximum chromosome aberrations were observed in 50 kR gamma rays and 40 mM EMS when compared to control (Plate – VII and VIII).

4.9. SDS-PAGE analysis

The SDS-electrophoresis patterns (Table-23; Plate-IX) showed that the number of polymorphic bands among the different treatments induced changes in protein pattern. The protein bands having molecular weights from 14.4 to 116.0 kDa. As a result of gamma irradiation, three bands disappeared with molecular weights 22.5, 35.0 and 62.5 kDa. Similarly, EMS treatments induced the protein band changes which disappeared two bands with molecular weights 19.0 and 40.0 kDa. And also EMS 20 mM induced one new band with molecular weight 49.0 kDa with the effect of mutagenic action.

4.10. RAPD Analysis

Five mutants were analysed to RAPD assay for identifying DNA polymorphism. Eight random decamer primers revealed a high DNA polymorphism among the mutant populations tall, dwarf, bushy, early maturity and bold seed mutants were analyzed. A total of 70 bands scored of which 40 bands were polymorphic with an average of 53.04 percentage polymorphism. Only four primers (OPS11, OPS17, OPC11 and OPC12) showed highest polymorphism and the primer PGF07 gave the lowest polymorphism (33.33%) (Table-24).
Based on the dendrogram constructed by Jaccard’s coefficient similarity in green gram mutants indicated two clusters; one comparing control and other cluster consist of tall mutant. The cluster of tall mutant sub-divided in to dwarf, bushy, early maturity and bold seed mutants. The bold seed mutant has lowest similarity with more variation from other mutant characters and control (Table-25 Fig.1).